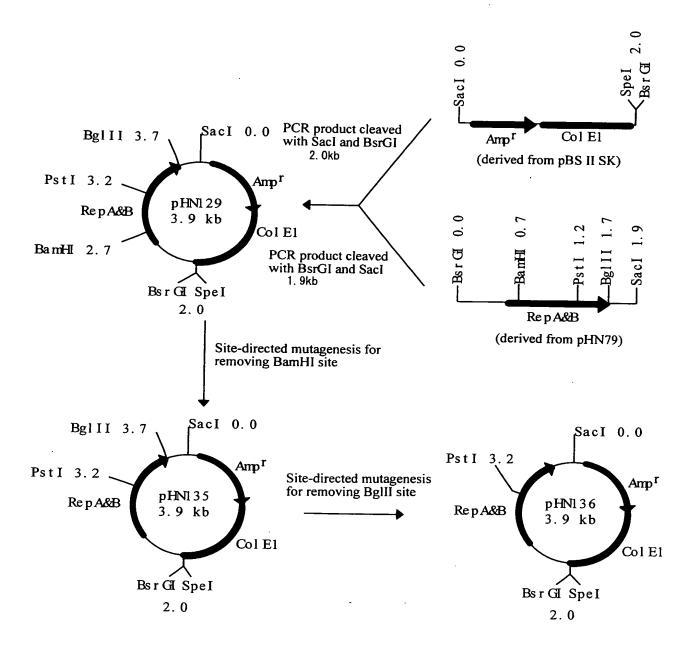
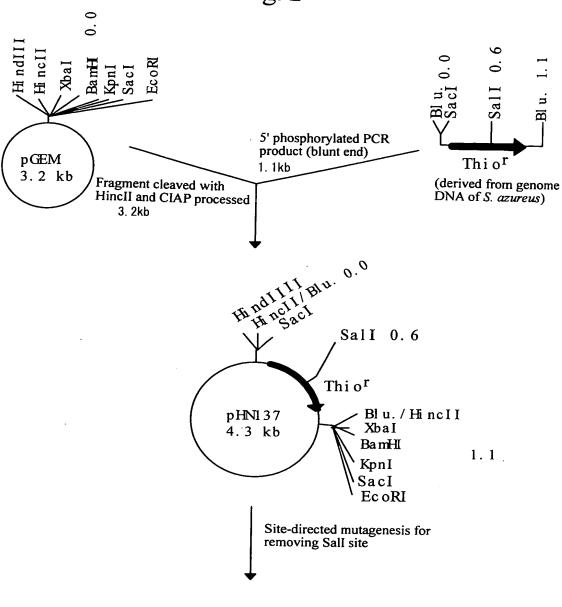
Fig. 1



Title: PROCESS FOR PRODUCING RECOMBINANT PROTEIN
IN BACTERIUM BELONGING TO THE GENUS
RHODOCOCCUS

Inventor(s): Nobutaka NAKASHIMA, et al. DOCKET NO.: 081356-0253

Fig. 2



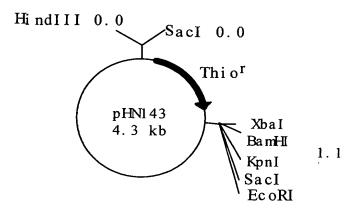


Fig. 3

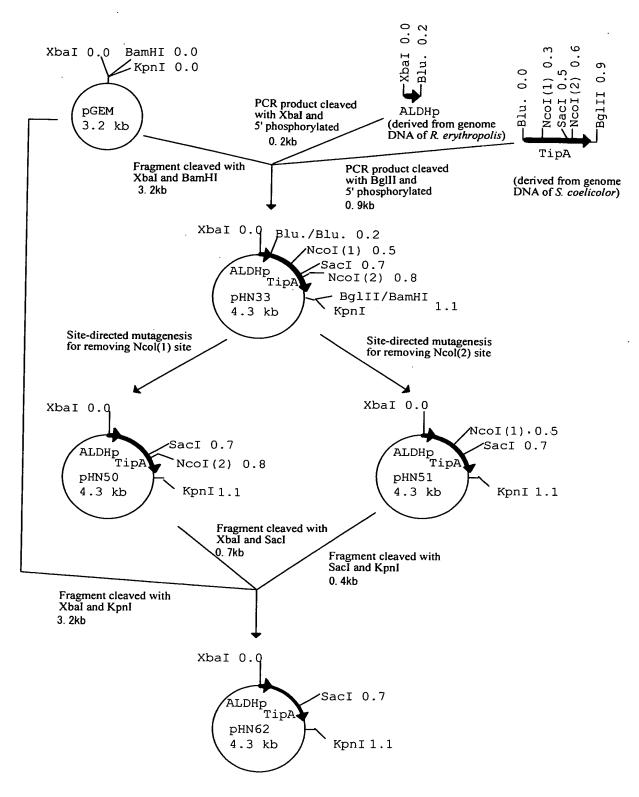
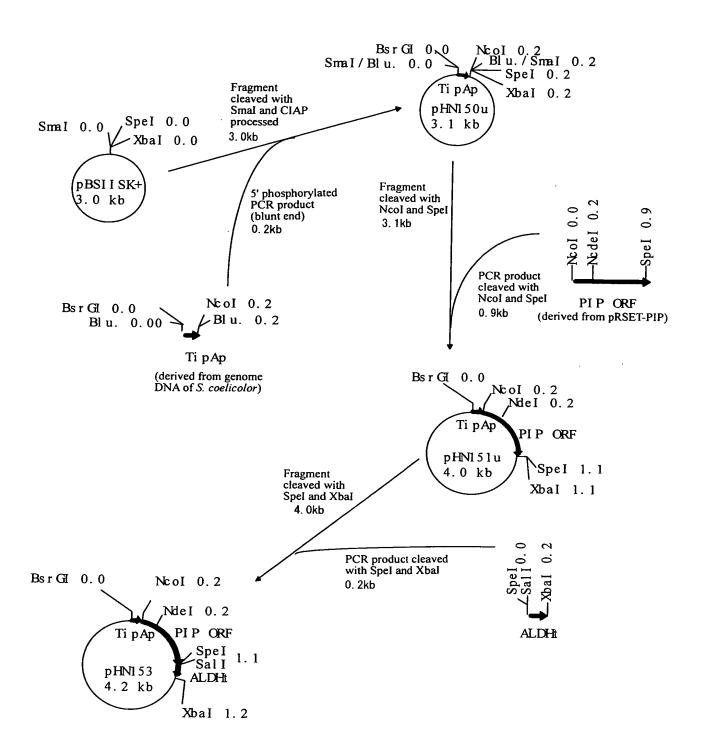
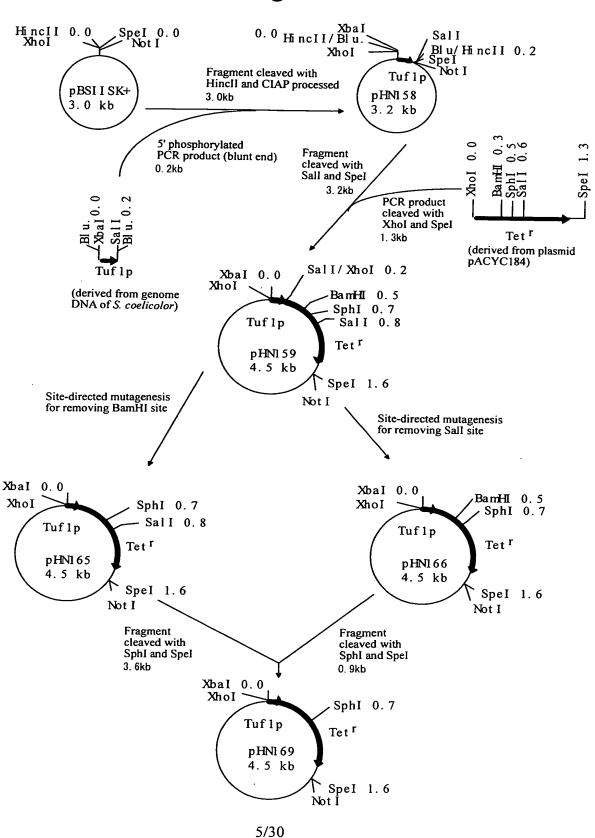


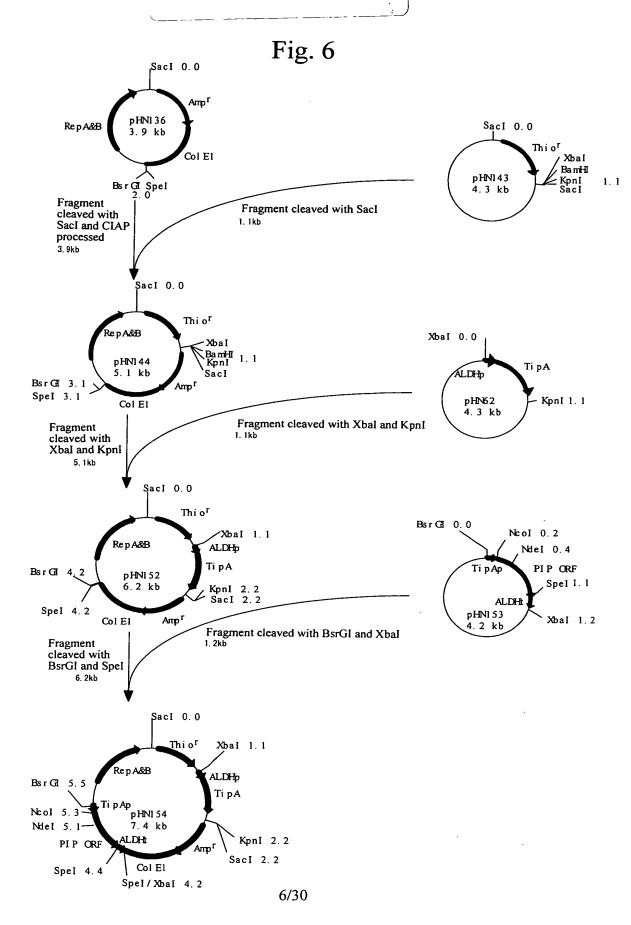
Fig. 4



Inventor(s): Nobutaka NAKASHIMA, et al. DOCKET NO.: 081356-0253

Fig. 5





Kpn I 3.8 Sacl 3.8 Thi o Sacl 0.0 pHNI71 9.0 kb ALDH Amp Fi pA- LG10p Ndel 6.6 PIP ORF Ncol 6.8 Bsr G 7.0 SpeI 6. 0 PCR product cleaved with BsrGI and Ncol (derived from plasmid pHN170) Fragment cleaved with BsrGI and Ncol 8.8kb NcoI 0.2 Spel 1.6 Ti pA-LG10p Tet ^r BsrG 0.0 pHN169 4.5 kb XbaI 0.0 Xbal/Spel 2.7 Fragment cleaved with Xbal and Spel 1.6kb XbaI 1.1 KpnI 2.2 Sacl 2.2 Tet ^r ALDH Xba [1.1 ALDIT Ti pA Thi o^r SacI 0.0 Amp Sac I 0.0 Thio Spel/Xbal 4.2 pHNI 70 9. 0 kb Ti pAp pHNI S4 7.4 kb Col El Col El RepA&B Ті рАр Fragment cleaved with Xbal and CIAP processed 7.4kb PIP ORF Spel 4.4 PIP ORF No. 1 5.34 Ncol 6.8 Ndel 6.6 — Bsr G 5.5 Bsr G 7.0 Spel 6.0

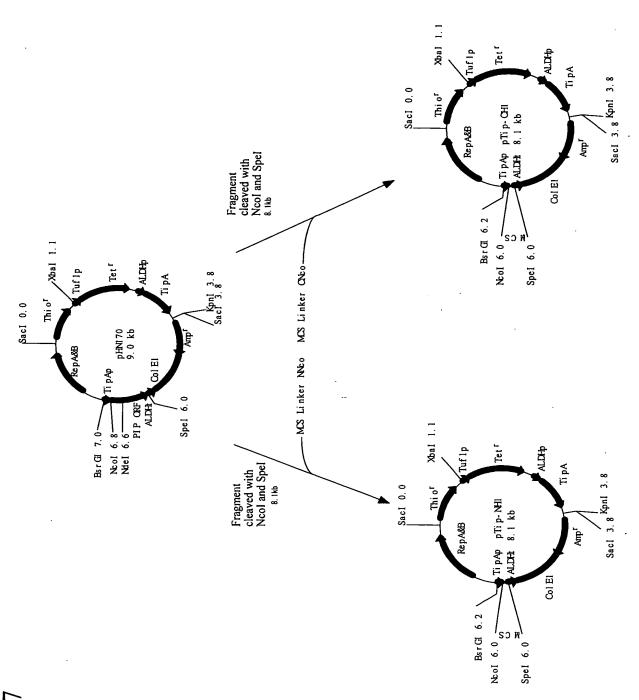
Xbal 1.1

Tet r

ALDH Ti pA

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Fig. 6 (continued)



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m Fig.}^{'}$

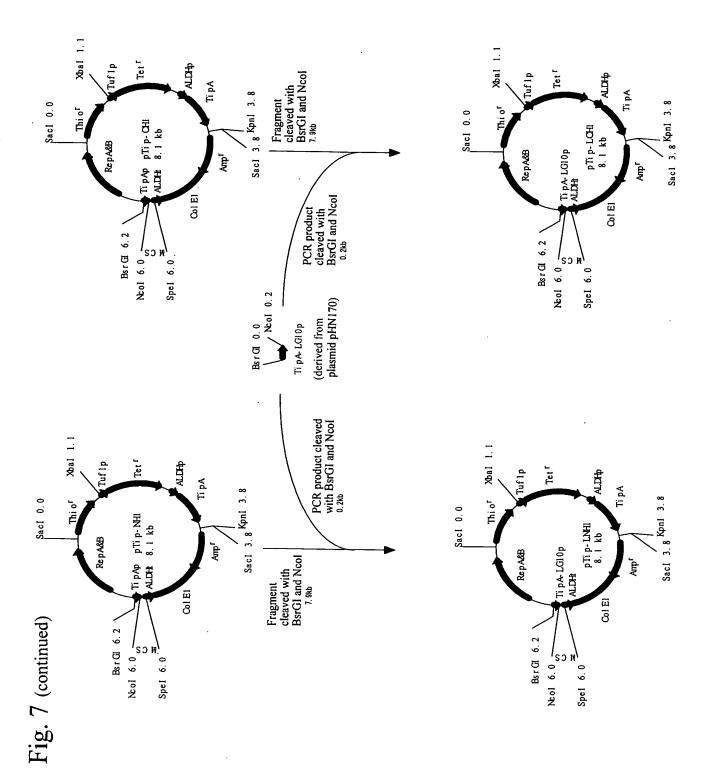
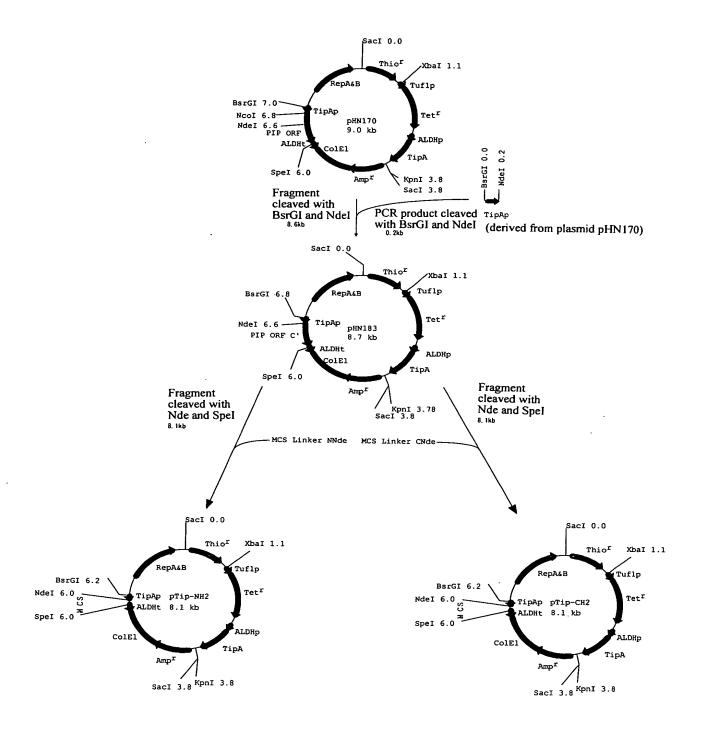
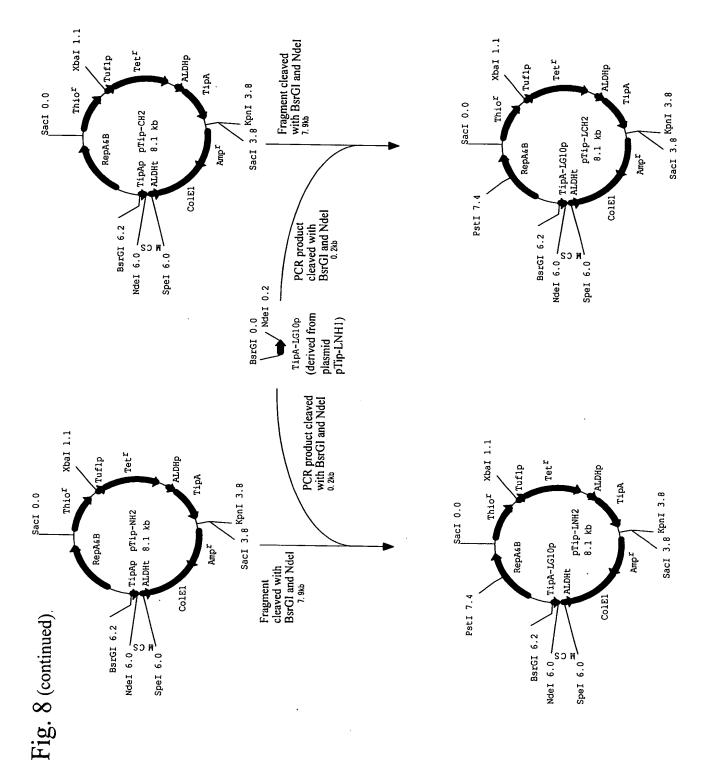


Fig. 8





ΥQ Tet ALDHp p T ip vector 8.1Kb Amp Co E1 SO RepA&B pT ip-CH2 (6 xH is) (Stop) Bam H J H ind III SnaB EcoR | BgⅢ Xhol Ndel Notl Spel ALDHŧ Sall pT ip-CH1 (8 XH is) (Stop) H ind III Bam H EcoR 1 SnaBl ioΑp N co l Bg⊞ Not Xhol Spel Sall (8 XH is) pT ip 小H2 Bam H | III pui II (Stop) N co I EcoR I SnaBl Ndel iоАр Not! Bg III Xhol Spel Sall pT ip →N H 1 (8 XH is) III pu III Bam H J (Stop) DA p EcoR | SnaBl Ncol Ndel Not BgⅢ Xhol Spel ALDHt Sall pT io LCH2 (6 xH is) Bam H | III pui II (Stop) **EcoR** I SnaBl Notl BgIII Ndel Xhol - p.A -G 1 0 p Spel ALDHt Sall pT ip ← CH 1 (8 XH is) Bam H I III pui II (Stop) SnaBl EcoR I ГрА -- G 10р N co l X ho l Thiostrepton induction system Noti BgIII ALDHt Spel Sall pT ip LNH2 (8 Hx 9) III pui H Bam H I (Stop) SnaBl EcoR | T ip A – _ G 1 0 p N co l Notl Ndel Bg Ⅲ Xhol ALDHt Spèl Sall pT ip LNH1 (6 xH is) Bam H I H ind III (Stop) SnaBl EcoR I LG10p Ndel N co l Bg III Notl Xhol Spel ALDHt

Luf1 p-I et' = transformation marker for R.erythropolis Am p^r = transformation marker for E. coliAntibiotic resistance marker Regions necessary for the autonomous Rep A & B = for R. erythropolis replication of a plasmid $Co \mathbb{E}1 = for E. coli$ ALDHt = transcription termination sequence ALD Hp = promoter which constitutively I pA ←G10p = improved TipA promoter produces TipA protein T pA = encodes a TipA protein T ip A p = Tip A promoter

Thio r = confers thiostrepton resistance

to R. erythropolis

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Fig. 9a

DOCKET NO.: 081356-0253

GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC GCC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CGC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG CAG CTC

ATG Met ¶co ¶co TAT AGA IGG ACG GOG TOA GAG AAG GGA AG RBS AGA **T**6T acé tca cet gag gag g<u>ca</u>

GGC CAC CAT CAC CAT CAC CAT ATG GGA ATT CTA CGT AGC GGC CGC GIV His His His His His Met Gly Ile Leu Arg Ser Gly Arg EcoRI

TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC Sa/I 66A 61y AGA TCT CGA (Arg Ser Arg (AAG CTT / Lys Leu /

CTC GCT GCG GGT GCG GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA

CAC ACG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCT

CCA CCC CGG TTG GTG ACC ACC GCT GGG GCT GAC CTG CTA CGC CGC CTC

AGC GGG ACT CTA GT

BSFGI GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC GGC ACG CGG CGC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG CAD CTC -35

ACG TCA CGT GAG GAG GCA GCG TGG ACG GGG TCA GAG AAG GGA GCG GCC ATG TRBS
GTC TAG AAA TAA TTT TGT TTA ACT TTA AGA AGG AGA TAT ACC

GGA ATT CTA CGT AGC GGC CGC GGA TCC AAG CTT AGA TCT CGA GGA CAT CAC GIY AFF GIY AFF GIY AFF GIY HIS HIS

CAT CAC CAT CAC TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC CTC His His His *

GCT

GCG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC ACG

3 GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT CCA CCC

G TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC AGC GGG

ACT CTA GT

DOCKET NO.: 081356-0253

GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CGC CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG

Met Mdel GTT TAA CTT TAA GAA GGA GAT ATA ACG TCA CGT GAG GAG GCA GCG TGG ACG GCE

GGC CAT CAC CAT CAC CAT CAC GCC ATG GGA ATT CTA CGT AGC GGC CGC GGA GIY His His His His His Ala Met Gly Ile Leu Arg Ser Gly Arg Gly Notl SnaBl EcaR Ncol

TCC AAG CTT AGA TCT CGA GGA TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG Ser Lys Leu Arg Ser Arg Gly * BanHI Hindlil Bg/I

CCC CTC GCT GCG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC

AAA CAC ACG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC

GTT CCA CCC CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC

CTC AGC GGG ACT CTA GT

GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CGC ACG GCG TGG CAC GCG GAA CGT CCG GGC <u>ITG CACI</u> CTC -35

ACG TCA CGT GAG GAG GCA GCG TCA GAG AAG GGA GCG CAT ATG RBS

G TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT

Ecori Snabi Noti Bamhi Hindii Bg/II Nool
GGA ATT CTA CGT AGC GGC CGC GGA TCC AAG CTT AGA TCT CGA GGA CAT CAC GIY Lie Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Arg Gly His His

CAT CAC CAT CAC TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC CTC GCT

GCG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC ACG

G GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT CCA CCC

CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC AGC GGG

ACT CTA GT

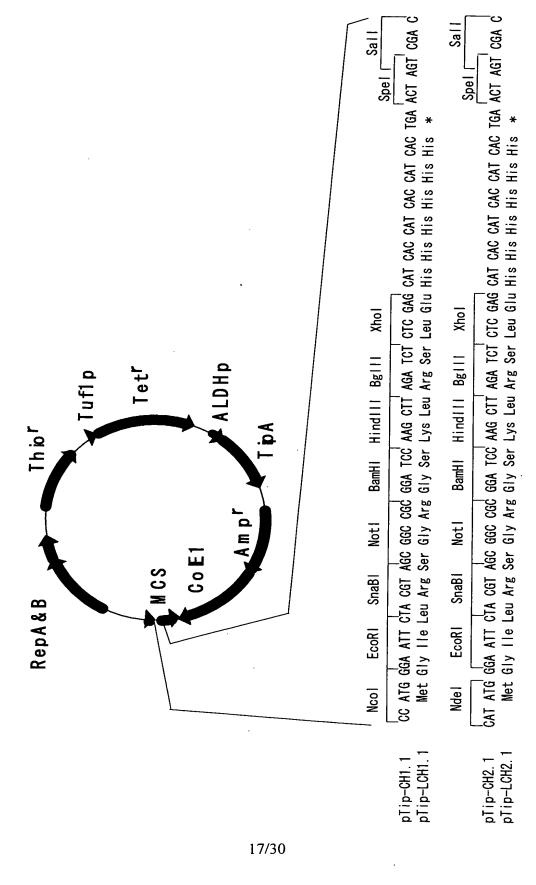


Fig. 1

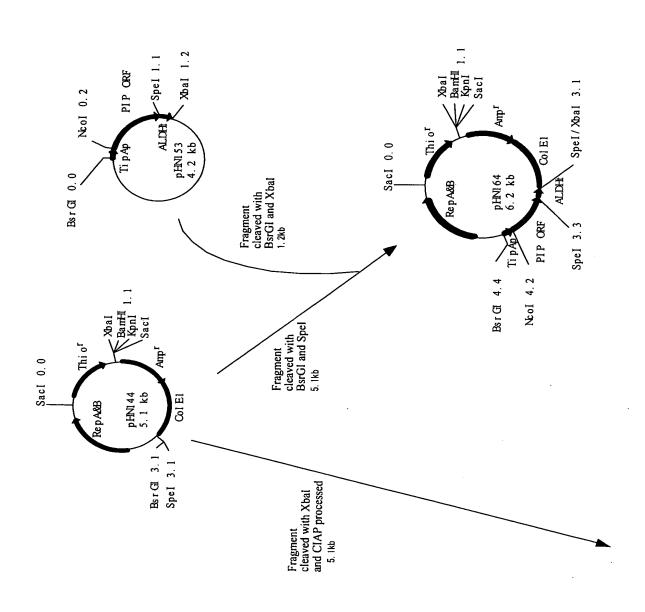
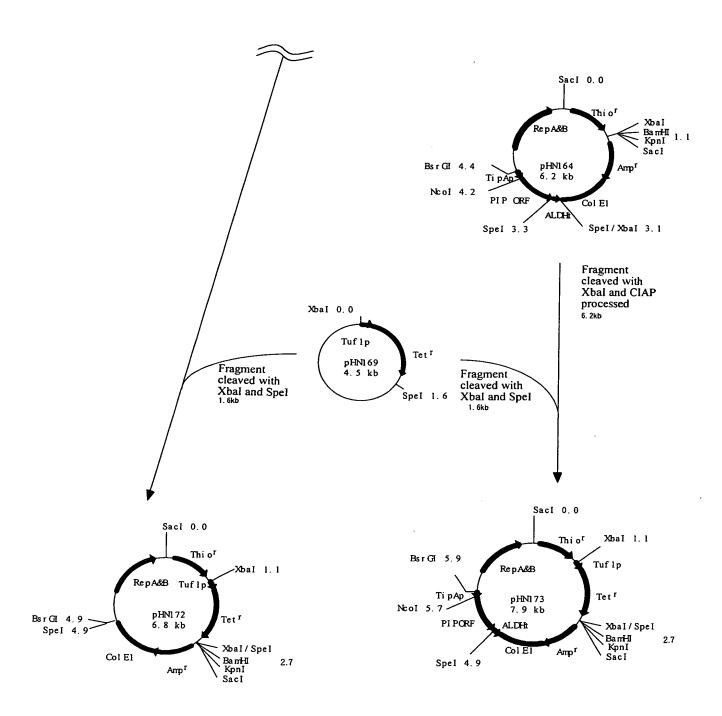


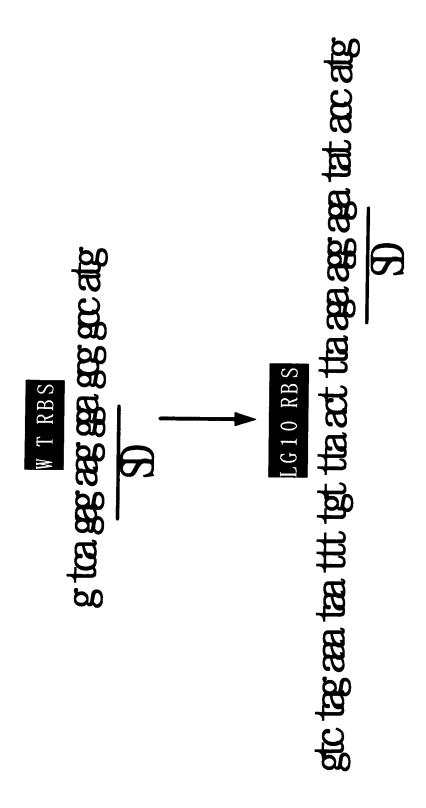
Fig. 1

Fig. 11 (continued)



cgcccgggctgagggagccgacggcacgcggctcac ggcgtggcacgcggacgtccgggcttgcacctcacgtc acgtgaggaggcagcgtggacggcgtcagaggaggagc -35 ggccatg

Fig. 12



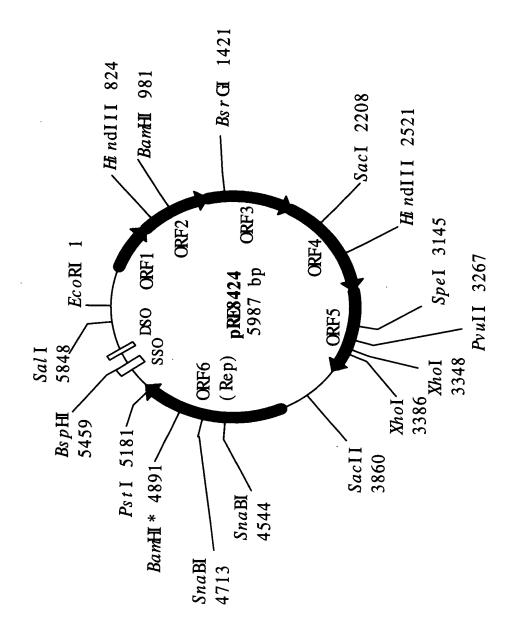


Fig. 14

Title: PROCESS FOR PRODUCING RECOMBINANT PROTEIN IN BACTERIUM BELONGING TO THE GENUS RHODOCOCCUS

Inventor(s): Nobutaka NAKASHIMA, et al. DOCKET NO.: 081356-0253

•		
Motif III	l a XYXXKXq X	53 LAAYLTKI AS 67 I GNYVSKNQT 54 MATIALAKGNS 80 LI BYLTKNQD 69 LABYI AKTQD 69 LABYI AKTQD 69 LABYI AKTQD
		53 67 54 80 69 69
Motif II	g XXg XXr a Xe Xt Xg XXn GwHXHXh XI X	GCDGYVRAVEI THGK- NGWHVHVHALL GLVGYVRANEI THGK- HGWHVHSHVLI VEHIYSDYEVTDS WA- NGWHLHRNMLL GYI GWRAAEVTRSKKNGYHPHLNLLV GYVGM RATEVTVGQI NGWHPHI HAI V GYVGM RATEVTVGQI NGWHPHI HAI V
		33 34 77 59 59
Motif I	Xvt XTXRH	MATMIMRH 33 MATLIQRH 33 MFVGIVRH 34 LVIFTARH 77 LVIFTARH 59 LVIFTARH 59 LVIFTARH 59
		26 27 76 27 27 27
Motif IV	GLXXCGXXWCPXC	GLRSCCKGW CPCC GLHTCCSVWACPVC GLVRCCRI WFCPEC GLMCCRI W.CPVC GLMCCRI W.CPVC K** ** ** **
		68 138 38 20 20
	Consensus	pRE8424 pAP1 pBL1 pJ V1 p1 J 101 pSN22

terminal moti

Wke y EXa XXgr Ra i XWYr gl r	276 WREFEFGSMÆRAI AWSRŒLR	WKEYEKASFGRRAL TWSKGLR	WREYEVGSKNLRS-SWSRGAK	WAQYEEALAGRRAI EWIRGLR	WIEYERATRGRRAI EWIRYLR	288 WIEYERATKGRRAI EWIRYLR	· · · · · · · · · · · · · · · · · · ·
	276	365	250	352	288	288	
Consensus	pRE8424	pAP1	pBL1	pJ Vl	pIJ101	pSN22	

CAGGIATGC-G-GA-AAACTITT--IXGGAACAA--GAAATAGAA-GTGA-AGAGEDCTAAGGAACCGCACTGGGAAAAAAGGGA--IGGGET---IXGGTAAGGGT
GAGGAAAA-GCGA-AGAGGTT--GGGAAAGAAGACGGAAAAGCGTGTCGGGGTT--GGGAAAGAAAGACGGAAAAGCGTGTCGGGGTT--GGGAAAGAAANicking site

CGAIREGENAGGG-[MA- GAGGG]----MAGETGGGGGAG-

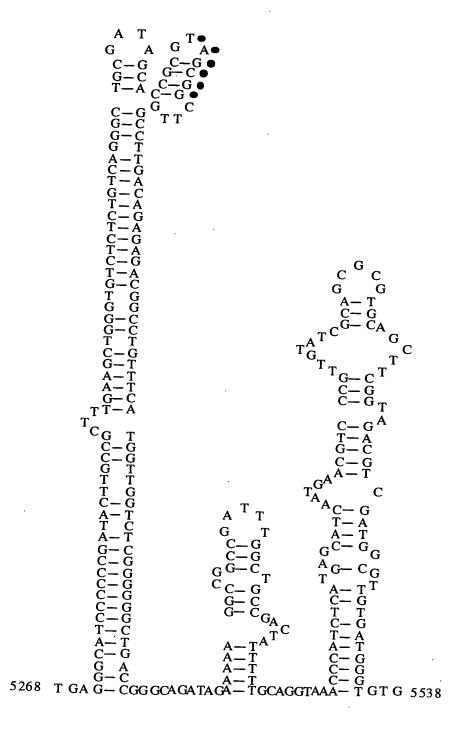
pRE8424

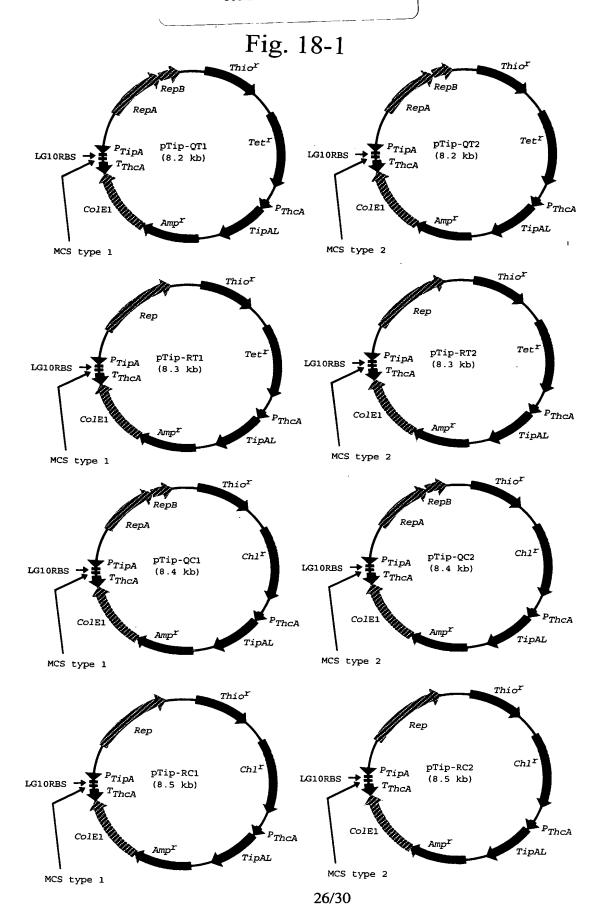
 $p\,AP\,1$

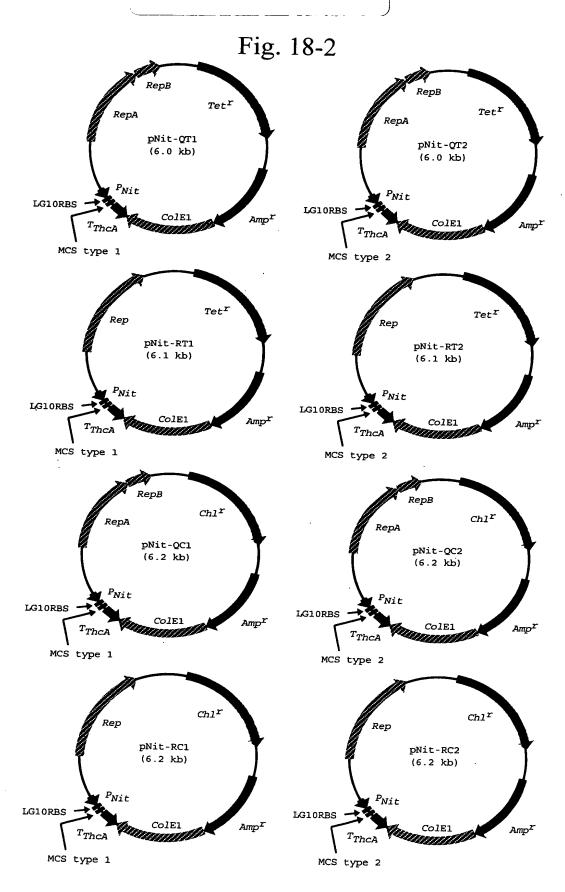
pBL1 pJV1 DS

Fig. 16

Fig. 17







Title: PROCESS FOR PRODUCING RECOMBINANT PROTEIN IN BACTERIUM BELONGING TO THE GENUS RHODOCOCCUS

Inventor(s): Nobutaka NAKASHIMA, et al DOCKET NO.: 081356-0253

TG TAC ATA TOG AGG COG CCT CCC AGG CCC CCG CCT CAG GCA CCC CAC CCC ACG CCG CCG CTC ACG CCG TCG CCG CAC CCG CAA CGT CCG CCC Ti pA-LG10p or Nit-LG10p

Fig. 19

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MCS

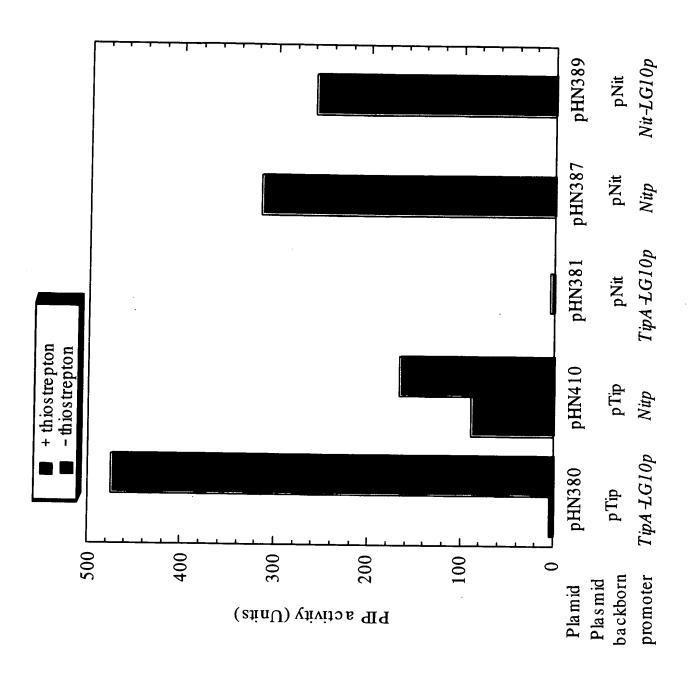
OC ATG GOC CAC CAT CAC CAT CAC CAT ATG GGA ATT CTA GGT AGC GGC GGA TOC AAG CTT AGA TCT CTC GAG CAT CAC CAT CAC CAT CAC TAGA Gly His His His His His Met Gly Ille Leu Arg Ser Cly Arg Gly Ser Lys Leu Arg Ser Leu Glu His His His His His His H ndIII BglII H ndIII Bg/III Bant Bant Not I Not I SnaBI SnaBI EcoRI Ncol Ty pe Me t Ty pe Ncol NdeI

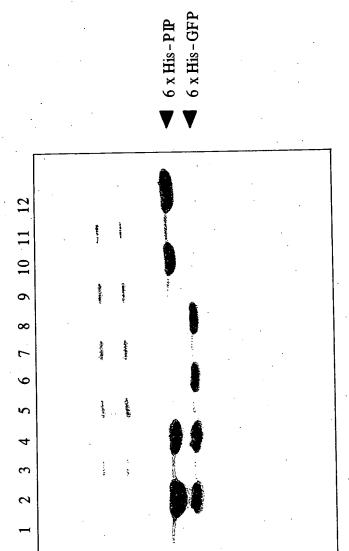
ALDHt

Spel Sall

ACT AGT CGA CCC ACC GCC ACC CGT GAG CCC CTC GCT GCG GGT GCC GGT GCG AAC TGC AAC ACG CCA AAC CTG CAC AAA CAC ACG GAG GTT GGA ATG AGC GCC ACG GAC ACA GCC GAT ACC GGC GCT GCA CCC GGT TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC GCC ACC

CAG ACT CTA GT





97 – – 99

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